Free Radical Biology and Medicine 98 (2016) 2–12

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Going retro: Oxidative stress biomarkers in modern redox biology

N.V. Margaritelis [a,e], J.N. Cobley [b], V. Paschalis [c,d], A.S. Veskoukis [a], A.A. Theodorou [d], A. Kyparos [a], M.G. Nikolaidis [a,*]

[a] Department of Physical Education and Sports Science at Serres, Aristotle University of Thessaloniki, Agios Ioannis, 62110 Serres, Greece
[b] Division of Sport and Exercise Sciences, Abertay University, Dundee, UK
[c] Department of Physical Education and Sport Science, University of Thessaly, Karies, Trikala, Greece
[d] Department of Health Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus
[e] Intensive Care Unit, 424 General Military Hospital of Thessaloniki, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
8 January 2016
Accepted 2 February 2016
Available online 17 February 2016

Keywords:
Biomarkers
Oxidative stress
Redox signaling
Physiology
Pathology

ABSTRACT

The field of redox biology is inherently intertwined with oxidative stress biomarkers. Oxidative stress biomarkers have been utilized for many different objectives. Our analysis indicates that oxidative stress biomarkers have several salient applications: (1) diagnosing oxidative stress, (2) pinpointing likely redox components in a physiological or pathological process and (3) estimating the severity, progression and/or regression of a disease. On the contrary, oxidative stress biomarkers do not report on redox signaling. Alternative approaches to gain more mechanistic insights are: (1) measuring molecules that are integrated in pathways linking redox biochemistry with physiology, (2) using the exomarker approach and (3) exploiting -omics techniques. More sophisticated approaches and large trials are needed to establish oxidative stress biomarkers in the clinical setting.

1. Introduction

Oxidative stress biomarkers have been used in the free radical field from its inception. For example, in their 1982 paper, Kelvin Davies and Lester Packer measured a biomarker of lipid peroxidation, in what is probably the most influential paper in the free radical and exercise field [1]. Even today, some of the most important exercise studies have used several oxidative stress biomarkers in order to substantiate the usefulness or uselessness of antioxidant supplementation (e.g., [2–4]). This supports the idea that the field of redox biology is inherently intertwined with oxidative stress biomarkers.

In the first experiments, oxidative stress biomarkers were useful in establishing the presence or absence of oxidative stress in various physiological processes and diseases [5]. This is a task that several available oxidative stress biomarkers can certainly fulfill. As the field of redox biology was progressing, the experimental questions concerning the nature and the consequences of the redox perturbation accompanying exercise have been refined. For example, efforts are now being made for unraveling the exact redox mechanisms through which exercise can ameliorate the negative consequences of a disease (e.g., [6]). Now, and in the past, oxidative stress biomarkers have been often used as a tool to reveal redox mechanisms. This actually implies that the oxidative stress biomarkers feature the required biochemical characteristics to report on redox signaling. However, oxidative stress biomarkers do not provide mechanistic insights [7], because they were not made for that purpose. In fact, the molecular understanding of the role of redox biochemistry in health and disease requires the precise identification of the modifying species, the biomolecular targets involved, the type of modification, the specific residue (s) modified, the reversibility of the oxidative modification and the cellular/organelle compartment that this process takes place [8].

Therefore, an intriguing question is what might be the true value of oxidative stress biomarkers in the modern redox biology era. To answer this question and demonstrate the plausible areas of application of redox biomarkers towards linking reactive species with physiology and pathology, we draw examples from exercise physiology. The main aim of this review is to highlight the applications, misuses and limitations of oxidative stress markers in modern redox biology. A secondary aim is to emphasize currently available mechanistic biomarkers that look promising and suggest specific steps that have considerable potential to progress current understanding. Our analysis indicates that oxidative stress biomarkers have several salient applications: (1) diagnosing oxidative stress, (2) pinpointing likely redox components in a physiological or pathological process and (3) estimating the severity, progression and/or regression of a disease (Fig. 1).

* Corresponding author.
E-mail address: nikolaidis@auth.gr (M.G. Nikolaidis).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.02.005
0891-5849/

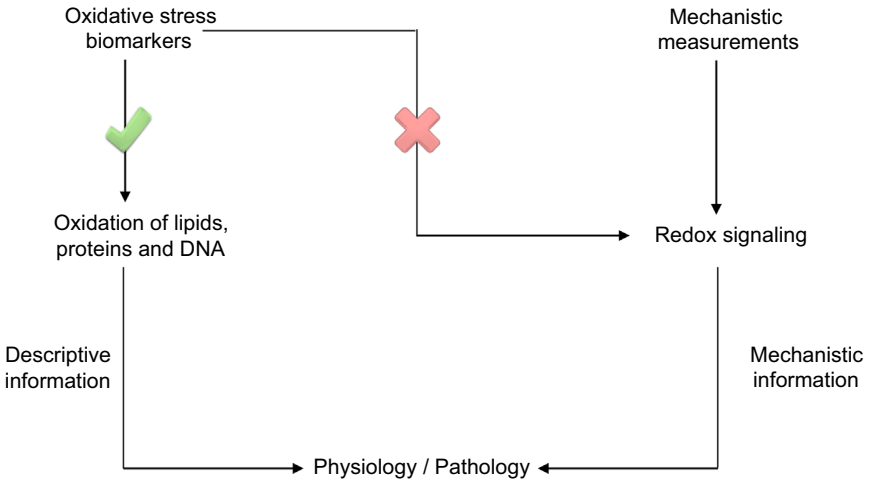

Fig. 1. Applications of oxidative stress biomarkers. Oxidative stress biomarkers are advantageous and practical for confirming the presence of oxidative stress and for providing a descriptive view of redox-associated processes linked to physiology and pathology. This descriptive view includes the recognition of a potential redox component of the process and the estimation of the severity, progression and regression of a disease. On the contrary, oxidative stress biomarkers do not convey mechanistic information, since they lack the mandatory specificity and selectivity required for such a purpose. Thus, they cannot report on redox signaling processes.

2. Applications of oxidative stress biomarkers

In biomedical research, a biomarker is defined as "*a characteristic that can be objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention*" [9]. The diagnostic potential of a biomarker depends, therefore, on the accurate distinction of the alterations that correspond to the onset, progression, regression, and ideally, to the prediction of a specific biological process at the time of specimen collection. On this basis, biomarkers are regarded as an integral part of molecular epidemiology aiming to fulfill the largely unmet need to integrate biochemical with translational and clinical research [10–12]. To this aim, emerging technologies, including proteomics, lipidomics, metabolomics, transcriptomics, genomics and real-time imaging have been implemented and have widely facilitated the identification of such biomarkers [13–16]. Along with these analytical advances, detailed criteria for an ideal biomarker have been proposed, briefly summarized as follows: (1) high sensitivity and accuracy, (2) high reproducibility, (3) biological plausibility with the investigated phenotype, (4) opposite responsiveness after a therapeutic treatment and (5) non-invasive collection and easy handling [16–22]. A noteworthy observation is that the potential to disclose the exact underlying mechanism of the investigated condition is not included in the proposed criteria of the ideal biomarker. This emphasizes that the biomarkers were not developed for unraveling mechanisms, but instead, for providing an integrative view of the biological process/disease under study.

Correspondingly, to date several oxidative stress biomarkers reliably reflect the exposure to an oxidant insult. On behalf of this objective, great analytical advances have been made, epitomized by the improvement in lipid peroxidation and protein oxidation assessment. In particular, and with regard to lipid peroxidation, less reliable and outdated approaches (e.g., the TBA test) have been replaced by sophisticated state-of-the-art techniques (e.g., MS-based methods), which infer non-enzymatic free radical-mediated lipid peroxidation in a more accurate way [23,24]. Likewise, great progress has been achieved in protein oxidation assessment. More specifically, the crude measurement of carbonyl groups that may also derive from diverse redox-unrelated processes (i.e., protein glycation by sugars or binding of aldehydes to proteins) is gradually being displaced by improved techniques that identify specific oxidized/nitrated amino acids, such as 3-nitrotyrosine and oxidized tryptophan. These techniques predominately involve mass spectrometry, in combination with gas chromatography (GC–MS, GC–MS/MS) or liquid chromatography (LC–MS/MS) [24,25].

When referring to redox biomarkers it is important to distinguish the biomarkers that offer a descriptive view of the general redox state of the organism from those that provide a more mechanistic read-out. By definition, oxidative stress biomarkers belong to the former category, providing a global snapshot of lipid peroxidation, protein oxidation or DNA oxidation according to the biomarker. On the contrary, they cannot be regarded as mechanistic biomarkers, because they are not integrated into a specific redox pathway and do not regulate redox signaling in a canonical manner (see Section 2.2). Below, we present selected examples of how oxidative stress biomarkers may be applied to exercise and clinical exercise physiology and pathology.

2.1. Physiology

Exercise is probably one of the most characteristic examples demonstrating that reactive species and oxidative stress are not necessarily "harmful" entities. In fact, regular exercise leads to many beneficial redox-related and redox-mediated adaptations, which are accompanied by repeated episodes of reactive species production [26,27]. With regard to the redox-related adaptations, it is well-established that exercise training results in: (i) increased levels of non-enzymatic antioxidants (e.g., glutathione), (ii) up-regulated gene expression and increased activity of the antioxidant enzymes (e.g., superoxide dismutase), (iii) increased molecular chaperone content (e.g., heat shock proteins), and (iv) decreased amount of oxidative stress biomarkers at rest (e.g., F_2-isoprostanes) [28–32]. Remarkably, when vitamin C was used along with a chronic exercise protocol, hampered redox-related adaptations were found in muscle (e.g., decreased expression of superoxide dismutase and glutathione peroxidase; [33]).

From a functional perspective, the aforementioned changes increase protection against exercise-induced oxidative stress. From a molecular perspective, these changes are regulated by several redox sensitive transcription factors, such as NF-κB, AP-1, HIF-α, PGC-1α, p53, HSF1 and Nrf2, which activate cyto-protective, angiogenic, metabolic and mitochondrial gene clusters [34–37]. The repeated induction of the aforementioned gene clusters underpins several exercise adaptations, such as mitochondrial biogenesis, angiogenesis, muscle hypertrophy, O_2 uptake and insulin